Remarks

Reconsideration and withdrawal of the objections to the specification and rejections of the claims, in view of the amendments and remarks herein, is respectfully requested. Claims 1-2 are amended. Claims 1-33 are pending.

In response to the finality of the Restriction Requirement and election of species requirement, Applicant reserves the right to petition the Commissioner to review the Restriction Requirement and/or the election of species requirement.

The amendments to the specification address the objections at page 2 of the Office Action.

The 35 U.S.C. § 112, Second Paragraph, Rejection

The Examiner rejected claims 1 and 8-9 under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. This rejection, as it relates to the pending claims, is respectfully traversed.

The Examiner alleges that the word "hydrolytic" in claim 1 is grammatically incorrect. The amendment to claim 1 renders the § 112(2) rejection of claim 1 moot.

The Examiner also alleges that the phrase "A-DNA, B-DNA, or Z-DNA" in claim 8 and the word "engrailed" in claim 9 are indefinite. It is Applicant's position that the words "A-DNA," "B-DNA," "Z-DNA" and "engrailed" are conventionally used in the art. Evidence that A-DNA, B-DNA and Z-DNA are terms conventionally used in the art is found at page 96 of the 1977 edition of Biochemistry: The Chemical Reactions of Living Cells (Metzler, ed., Academic Press, Inc., New York, NY (1977)) and the titles in Sponer et al. (Biophys. J., 73, 86 (1997)) and Gruskin et al. (Biochemistry, 32, 2167 (1993)) (a copy of each is enclosed herewith). At page 96 of Biochemistry: The Chemical Reactions of Living Cells, it is disclosed that in the A form of DNA, the base pairs are inclined to the helix axis by about 20°, while in the B form of DNA, the base pairs lie almost normal to the axis. Moreover, the title in both Sponer et al. and Gruskin et al. refers to B-DNA and Z-DNA ("Base-base and deoxyribose-base stacking interactions in B-DNA and Z-DNA: a quantum-chemical study" and "B-DNA to Z-DNA structural transitions in the SV40 enhancer: stabilization of Z-DNA in negatively supercoiled DNA minicircles," respectively).

With respect to "engrailed," the Examiner is requested to consider the title of a 1990 article authored by Kissinger et al. (Cell, 63, 579 (1990), of record)): entitled "Crystal Structure of an Engrailed Homeodomain-DNA Complex at 2.8 Å Resolution: A Framework for Understanding

Homeodomain-DNA Interactions" (emphasis added).

Therefore, "A-DNA", "B-DNA", "Z-DNA" and "engrailed" are terms that are conventionally used in the art. Accordingly, withdrawal of the 35 U.S,C. § 112, second paragraph, rejection is respectfully requested.

The 35 U.S.C. § 101 Rejection

The Examiner rejected claims 1-2, 4-18 and 27-29 under 35 U.S.C. § 101 as not being supported by either a specific asserted utility or a well established utility. This rejection is respectfully traversed.

Applicant's invention includes a synthetic (artificial) peptide or polypeptide that has the specificity of a DNA binding protein and the hydrolytic or redox activity of a metal binding domain, which synthetic peptide or polypeptide delivers a metal to a target DNA that is bound by the DNA binding domain, resulting in cleavage of the target DNA, i.e., the synthetic peptide or polypeptide is a nuclease (page 3, lines 5-9 of the specification). Therefore, Applicant's specification provides a specific asserted utility for the claimed synthetic peptide or polypeptide.

In addition, Applicant's specification discloses that a number of different approaches had been employed to prepare artificial nucleases having sequence specificity. Those approaches included linking a nucleic acid binding domain from one protein to a domain from another protein that exhibits nuclease activity, linking an oligonucleotide that binds to a specific nucleotide sequence in a target nucleic acid to a domain of a protein that has nuclease activity, and linking an oligonucleotide to metal and organic complexes that cleave nucleic acid (page 1, lines 14-28 of the specification). See, for example, Kim et al. ("Hybrid restriction enzymes: zinc finger fusions to FokI cleavage domain", Proc. Natl. Acad. Sci. USA, 93, 1156 (1996)), Zuckerman et al. ("Site-specific cleavage of structured RNA by a staphylococcal nuclease-DNA hybrid", Proc. Natl. Acad. Sci. USA, 86, 1766 (1989)), and Matsumura et al. ("Lanthanide Complex-Oligo-DNA hybrid for sequence-selective hydrolysis of RNA", J. Chem. Soc. Chem. Comm., 2019 (1994)) (all of record). Thus, as the art clearly recognized the utility of artificial nucleases, Applicant's synthetic peptide or polypeptide has a well established utility.

Hence, withdrawal of the § 101 rejection is respectfully requested.

The 35 U.S.C. § 112, First Paragraph, Rejections

The Examiner rejected claims 1-2, 4-18 and 27-29 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention (a written description rejection) and in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention (an enablement rejection). These rejections are respectfully traversed.

The first basis for the § 112(1) rejections is that the claimed invention is not supported by either a specific asserted utility or a well established utility, and so one skilled in the art would not know how to use the claimed invention. However, as discussed above, Applicant's invention is supported by both a specific asserted utility and a well established utility.

The other bases for the § 112(1) rejections are that: 1) reference to the units in Table 1 ($k=10^{-6}sec^{-1}$) at page 55 of the specification appears to contradict the units in Table 1 (rates (K_{obs}) (s^{-1} x 10^7); 2) the meaning of "10/10", "25/25" and "50/50" in the first column of Table 1 is unclear; 3) the difference between data in column 3 and column 4 in Table 1 is unclear; 4) the cleavage of BNPP by a synthetic endonuclease is not the cleavage of a nucleic acid by that endonuclease; 5) the meaning of A_{43}-$R_{(19)}$, Q_{44}-$E_{(20)}$, and T_{27}-L_{34} and E_{42}-K_{57} at pages 50-51 of the specification is not apparent; 6) Figure 8 purportedly does not show that EuP_3 catalyzes the cleavage of supercoiled, double stranded DNA; 7) there is no disclosure that the DNA binding domain and the metal binding domain of the synthetic endonuclease are the same; 8) the synthetic peptide/polypeptide lacks specificity of nucleic acid cleavage; and 9) the specification fails to teach what portion of SEQ ID NO:3 is catalytically active.

The amendment to claim 2 renders basis 9 of the rejection moot.

With respect to 1), at page 55, lines 20-21 it is disclosed that the synthetic metallopeptide catalyzes BNPP hydrolysis with rate constants on the order of $k = 10^{-6}sec^{-1}$ (Table 1) (emphasis added), i.e., $1/10^6$sec. The fifth column of Table 1 ("Rates($K_{(obs)}$) (s^{-1} x 10^7)", i.e., $10^{-7}sec^{-1}$ or $1/10^7$ sec) has numbers ranging from 1.4 to 212.0, that is, 1.4 x 10^{-7}/sec to 2.12 x 10^{-5}/sec, a two log range on the order of $k = 10^{-6}sec^{-1}$. Thus, the statement on page 55, lines 20-21 is clear in view of the fifth column in Table 1.

To discern the meaning of "10/10", "25/25" and "50/50" in the first column of Table 1 (basis 2 of the rejection), the Examiner is pointed to the top of column 1, "Concentration (μm

Eu/μM P3)".

The terms "[EuP3]" and "[$EuP3_2$]" in column 3 of Table 1 refer to a EuP3 monomer and a singly occupied dimer, respectively (see page 48, lines 6-9 and page 54, lines 1-2 and 14-16 of the specification) (basis 3).

With respect to 4), the Examiner is requested to consider that an assay which employs BNPP (a substrate having a phosphodiester bond) is art recognized as an assay which measures hydrolysis of phosphodiester bonds, bonds found in nucleic acid molecules (see, e.g., Hegg et al., Inorg. Chem., 38, 2961 (1999), Berg et al., J. Comb. Chem., 1, 96 (1999), and Chand et al., Chemistry, 6, 4001 (2000) (a copy of each is enclosed herewith).

With respect to 5), the Examiner is respectfully requested to refer to page 50, lines 18-21 of the specification, where it is disclosed that the helix-turn-helix motif of the engrailed homeodomain encompasses residues 27-56 and page 50, line 29 of the specification where it is disclosed that $X_{(n)}$ denotes a numbering scheme. Thus, T_{27}-L_{34} and E_{42}-K_{57} refer to residue 27 to residue 34, and residue 42 to residue 57, respectively, in the engrailed homeodomain. Moreover, A_{43}→$R_{(19)}$ indicates an alanine to arginine substitution at position 19 in a synthetic peptide of the invention which corresponds to the alanine residue at position 43 in the engrailed homeodomain (see Figure 2). Similarly, Q_{44}→$E_{(20)}$ indicates a glutamine to glutamic acid substitution at position 20 in a synthetic peptide of the invention which corresponds to the glutamine residue at position 44 in the engrailed homeodomain.

Figure 8 shows an agarose gel having samples subjected to electrophoresis. The samples included pUC19 DNA alone (lane 1), pUC19 DNA and synthetic peptide (lanes 6-9), pUC19 DNA and metal/peptide (lanes 10-15, Eu:P3) or pUC19 DNA and metal (lanes 2-5, $EuCl_3$). It is disclosed that after 24 hours, the concentration-dependent formation of an open circular ("nicked", type II) from supercoiled, type I was observed (page 56, lines 9-10 of the specification). Moreover, Figure 8 clearly shows that the levels of supercoiled and open circular DNA in lanes 10-15 (lanes with target DNA and metal/peptide) decrease, i.e., they are cleaved.

In response to basis 7), the Examiner is requested to consider that the specification discloses that the DNA binding domain and the metal binding domain are substantially superimposable in their helix orientation, and so such a metal binding domain can be introduced to a DNA binding domain, e.g., by replacing the turn or loop in the DNA binding domain with the metal binding domain (page 3, lines 19-23).

Lastly, with regard to specificity (basis 8), the Examiner is requested to note that engrailed binds to a consensus sequence TCAATTAAAT (the underlining denotes the most conserved positions, see Desplan et al., Cell, 54, 1081 (1988), a copy is enclosed herewith), and pUC19 has two CAATTAA sequences.

No

It is respectfully submitted that the pending claims are in conformance with § 112(1). Hence, withdrawal of the § 112(1) rejection is appropriate and is respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-373-6959) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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Date January 10, 2003 By _____
Janet E. Embretson
Reg. No. 39,665

CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 10 day of January, 2003.

Candis B. Buending
Name

_____
Signature

CLEAN VERSIONS OF AMENDED SPECIFICATION PARAGRAPHS

Please replace the paragraph beginning at line 24 on page 8 with the following paragraph:

C1

Figure 1C. Two views of the overlay of engrailed (1ENH) helix-turn-helix (HTH) region (α2-α2) and one EF-hand of calmodulin (1OSA; third Ca-site) thus illustrating that the helices occupy the same space. The C-terminal α3 is the homeodomain recognition helix which binds in the DNA major grove. The Ca(II) ion is shown as a sphere.

Please replace the paragraph beginning at line 29 on page 8 with the following paragraph:

C2

Figure 2. Sequences of peptide P2 (control; SEQ ID NO:1) P3, P4, P4a and P5 (synthetic; SEQ ID NO:2, SEQ ID NO:7, SEQ ID NO:4, SEQ ID NO:3 and SEQ ID NO:5, respectively), and loop modified Engrailed (SEQ ID NO:6). Parent protein sequence is indicated by double or single underlining (homeodomain and EF-hand). Expected sites of Ca(II) and Eu(III) binding are indicated by an x and the 12 residues of the Ca-binding loop are shaded.

Please replace the paragraph beginning at line 29 on page 9 with the following paragraph:

C3

Figure 7. The top panel is a representative gel showing the disappearance of type I band (supercoiled pBR322 plasmid) as a function of increasing EuP3 (5-25 μM). This gel shift is not seen with free metal over the same concentration range (right lanes), or with the control peptide, P2. An attenuated shift is observed for free P3. The lower panel is a graph showing quantified volumes of type I band (arbitrary volume units) as a function of EuP3 concentration. Data are an average of three gel shift assays.

CLEAN VERSION OF PENDING CLAIMS

C4

1. (Amended) An isolated synthetic peptide or polypeptide comprising a domain which specifically binds a nucleic acid sequence and a domain which specifically binds a metal which is hydrolytically or redox active, wherein the domain which specifically binds the metal is within the domain which specifically binds the nucleic acid sequence.

2. (Amended) The peptide or polypeptide of claim 1 which comprises the amino acid sequence TERRRQQLDKDGDGTIDEREIKIHFQNKRAKIK (SEQ ID NO:2), or a portion thereof which binds the nucleic acid sequence and the metal.

3. (Previously Amended) The peptide or polypeptide of claim 1 which comprises the amino acid sequence TERRRFDKDGNGYISAAELRHVKIWFQNKRAKIK (SEQ ID NO:3), or a catalytically active portion thereof.

4. The peptide or polypeptide of claim 1 which comprises at least 20 residues.

5. The peptide or polypeptide of claim 1 which comprises a consensus EF-Hand sequence.

6. The peptide or polypeptide of claim 1 wherein the domain which specifically binds the nucleic acid sequence is a domain from a transcription factor.

7. The peptide or polypeptide of claim 1 wherein the domain which specifically binds the nucleic acid sequence comprises a helix-turn-helix motif, a relaxed helix-turn-helix motif, a winged helix-turn-helix motif, a helix-loop-strand motif, or a hormone receptor motif.

8. The peptide or polypeptide of claim 1 which binds dsDNA, dsRNA, ssDNA, ssRNA, A-DNA, B-DNA, or Z-DNA.

9. The peptide or polypeptide of claim 6 wherein the transcription factor is engrailed.

10. The peptide or polypeptide of claim 1 wherein the domain which specifically binds the nucleic acid sequence is a homeodomain.

11. The peptide or polypeptide of claim 6 wherein the transcription factor comprises a helix-turn-helix domain.

12. The peptide or polypeptide of claim 7 which comprises alpha-helices 2 and 3 of a helix-turn-helix motif.

(C4 cont)

13. The peptide or polypeptide of claim 1 wherein the hydrolytic metal is a transition metal.

14. The peptide or polypeptide of claim 1 which binds Zn(II), Cd(II), Ce(II), Cr(IV), Fe(III), Co(III), Mn(II) and Cu(II).

15. The peptide or polypeptide of claim 1 which binds a lanthanide.

16. The peptide or polypeptide of claim 1 which binds Ca(II) or Mg(II).

17. The peptide or polypeptide of claim 1 which binds Eu(III).

18. The peptide or polypeptide of claim 1 further comprising a protein transport domain.

19. An isolated nucleic acid molecule comprising a nucleic acid segment which encodes the peptide or polypeptide of claim 1, or a sequence complementary thereto.

20. An expression cassette comprising the nucleic acid molecule of claim 19 which is operably linked to a promoter functional in a host cell.

21. A host cell comprising the expression cassette of claim 20.

22. The host cell of claim 21 wherein the host cell is a prokaryotic cell.

23. The host cell of claim 21 wherein the host cell is a eukaryotic cell.

24. The host cell of claim 21 wherein the host cell is a plant cell.

cf (cont)

25. A vector comprising the expression of cassette of claim 20.

26. A method to identify a synthetic endonuclease comprising:

a) identifying a secondary structure in a first amino acid sequence that binds to a specific nucleic acid sequence which has geometrical similarity to the secondary structure of a second amino acid sequence that binds a metal; and

b) identifying a third amino acid sequence that comprises at least a portion of the first amino acid sequence and at least a portion of the second amino acid sequence to yield a synthetic endonuclease which specifically binds the specific nucleic acid sequence and specifically binds the metal.

27. A method of using a synthetic endonuclease specific for a particular nucleic acid sequence comprising: contacting a sample comprising isolated nucleic acid with an amount of the peptide or polypeptide of claim 1 effective to cleave at least one nucleic acid sequence in the sample.

28. A method to detect the presence of a nucleic acid sequence in a sample comprising:

a) contacting a sample comprising nucleic acid suspected of containing a nucleic acid sequence recognized by the peptide or polypeptide of claim 1, with the peptide or polypeptide of claim 1; and

b) determining or detecting whether the peptide or polypeptide cleaves the nucleic

acid sequence.

29. The method of claim 28 wherein the detection or determination is by gel electrophoresis.

30. (Previously Amended) The peptide or polypeptide of claim 1 which comprises the amino acid sequence TERRRFRVFDKDGNGYISAAEKIWFQNKRAKIK (SEQ ID NO:4), or a catalytically active portion thereof.

31. (Previously Amended) The peptide or polypeptide of claim 1 which comprises the amino acid sequence TRRRRFLSFDKDGDGTITTKEEVWFQNRRMKWK (SEQ ID NO:5), or a catalytically active portion thereof.

32. The peptide or polypeptide of claim 1 which comprises the amino acid sequence DEKRPRTAFSGEQLARLKREFNENRYLTERRRLRVFDKDGNGFISAAEKIWFQNK RAKIKKST (SEQ ID NO:6), or a catalytically active portion thereof.

33. The peptide or polypeptide of claim 1 which comprises the amino acid sequence TERRRQQLDKDGDGTIDEREIKIWFQNKRAKIK (SEQ ID NO:7), or a catalytically active portion thereof.